Case Report

Serum Cystatin C in the Estimation of Glomerular Filtration on Chronic Angiotensin-Converting Enzyme Inhibitor Therapy: An Illustrative Case Report

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A 45-year-old man with a 15-year history of hypertension was treated with angiotensin-converting enzyme inhibitor (ACEI) monotherapy for the past 8 years. On treatment, his current serum creatinine is 1.1 mg/dL and the estimated glomerular filtration rate (eGFR) is 79.4 mL/min/1.73 m^2 using the 4-variable Modification of Diet in Renal Disease (MDRD) equation. Does this patient have stage 2 chronic kidney disease (CKD) according to the eGFR? The answer is no, not by the eGFR alone. This patient had a normal urine albumin:creatinine ratio (6 mg/g) and has no history of kidney injury or damage. Because ACEIs reduce intraglomerular pressure by dilating both afferent and efferent arterioles, less creatinine is filtered from the blood into the urine, elevating the blood creatinine level. For this reason, cystatin C, an alternative measure of renal filtration, was assessed on the same blood draw and found to be 0.65 mg/L. Equations used to estimate eGFR from cystatin C generated values from 117.0 to 132.9 mL/min for this patient. Why was the eGFR using cystatin C approximately 40 mL/min higher than the creatinine-based measures? This paper will review the differences between serum creatinine and cystatin C as filtered proteins by the glomerulus and their further processing by the renal tubules. It is possible that cystatin C, based on its different properties, may be a more reasonable measure of renal filtration function in patients on drugs that block the renin-angiotensin system (RAS), particularly in the setting of normal renal function.

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CASE PRESENTATION

A 45-year-old Caucasian man presented with headaches 15 years ago and was found at age 33 to have a blood pressure level of 160/110 mm Hg. Findings of an evaluation at that time for secondary causes of hypertension with blood and urine testing, 24-hour urine collection, and renal magnetic resonance angiography were unremarkable. Over the following 15 years, the patient made consistent efforts to lose weight, reduce dietary salt and alcohol intake, and improve fitness. He was treated initially with amlodipine 10 mg po qd for 7 years and then was switched to ramipril 10 mg po qd for the next 7 years. Within the last year, he has been taking benazepril 40 mg po qd. Throughout this period the blood pressure has consistently been <130/85 mm Hg. His current physical examination results are unremarkable, and

Table I. Calculated eGFRs for the Same 45-Year-Old Male Caucasian Patient With Various Equations Based on Serum Creatinine (1.1 mg/dL) and Cystatin C (0.65 mg/L)

Equations Using Serum Creatinine	Creatinine eGFR Result (mL/min/1.73 m^2)	Equations Using Cystatin C	Cystatin C eGFR Result (mL/min/1.73 m^2)
Full 6-variable MDRD $170\times(Cr-0.9990\times(age-0.176)\times(BUN)^{-0.17}\times albumin^{0.318}\times(0.762,$ if female)$\times(1.18,$ if African American)	79.4	Arnal-Dade $74.835/(CysC^{1.333})$	132.9
4-variable MDRD $186\times(Cr)^{-1.154}\times age^{-0.203}\times 0.742$ (if female)$\times 1.210$ (if African American)	79.6	Rule $66.8\times(CysC)^{-1.30}$	117.0
Mayo Quadratic exp $(1.911+[(5.249/Cr)-(2.114/Cr^2)]-0.00686\times Age-0.205$ (if female)) If Cr <0.8 mg/dL, use 0.8 for Cr	102.2	MacIsaac $(84.6/CysC)-3.2$	127.0
Crockcroft Gault $(140-age)\times$weight (kg)/$(Cr\times 72)\times 0.85$ if female	79.1 (mL/min)	Tan $(87.1/CysC)-6.87$	127.1

Abbreviations: Cr, serum creatinine; CysC, serum cystatin C; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease.

body mass index is 22 kg/m^2. As part of usual care of the patient on an RAS-blocking agent, serum creatinine and other markers were measured. Laboratory test results were as follows: sodium, 138 mmol/L; potassium, 4.6 mmol/L; chloride, 105 mmol/L; carbon dioxide, 29 mmol/L; glucose, 97 mg/dL; blood urea nitrogen, 17 mg/dL; creatinine, 1.1 mg/dL (Jaffe method, Siemens Advia 2400, Beaumont Reference Laboratories, Royal Oak, MI); calcium, 10.2 mg/dL; phosphate, 3.6 mg/dL; uric acid, 8.0 mg/dL; and cystatin C, 0.65 mg/L (latex particle enhanced immunonephelometry, Dade Behring, Mayo Laboratories, Rochester, MN[1]). Creatine kinase was in the normal range at 183 U/L (normal, 40–230 U/L). eGFR calculated by the modified 4-variable MDRD equation was 76.9 and by the full 6-variable equation, 79.4 mL/min/1.73 m^2, 102.2 mL/min/1.73 m^2 by the Mayo quadratic method, and 79.1 mL/min by the Cockcroft-Gault formula for creatinine clearance (Table I).[2–4] The eGFRs calculated from cystatin C equations ranged from 117 to 133 mL/min/1.73 m^2 (Table I).[5–8]

DISCUSSION

Does this patient have National Kidney Foundation stage 2 CKD (eGFR, 60–89 mL/min/1.73 m^2) according to the eGFR? The answer is no, not by the eGFR alone. This patient had a normal urine albumin:creatinine ratio (6 mg/g) and has no history of kidney injury or damage. Accurate and efficient laboratory estimation of renal filtration function is essential for the screening and detection of reduced eGFR, which is a principal indicator of CKD. This is important, since CKD is recognized as a cardiovascular risk state that invokes patient and physician awareness and calls for additional diagnostic and therapeutic measures above those done for the general population. The limitations of serum creatinine as a blood measure reflecting renal filtration of blood have been extensively debated. Creatinine is a low–molecular weight (113 Dalton) protein derived from the breakdown of skeletal muscle creatine phosphate, which is used as an energy substrate. It is freely filtered by the glomerulus and also undergoes a small amount of tubular secretion. Highly dependent on muscle mass, creatinine levels vary greatly among individuals who differ in age, sex, diet, and race. The first practical test for the determination of creatinine in urine, described more than 100 years ago (1886) by Max Jaffe (1841–1911), involves the formation of the red–yellowish brown creatinine picrate by the bonding of picric acid and creatinine in an alkaline solution.[9] In 1936, the Benedict method of creatinine determination was reported; it involves the reaction of 3,5-dinitrobenzoic acid with creatinine in an alkaline medium.[10] Both of these reactions require a high pH, on the order of 12 or 13, in order to deprotonate the creatinine so that the

Table II. Comparison of Serum Cystatin C and Creatinine

	Cystatin C	Creatinine
Function	Extracellular cysteine protease inhibitor	Breakdown product of muscle creatine phosphate
Production	Produced by all nucleated cells	Derivative of creatine, produced in skeletal muscle
Size	13,250 Daltons	113 Daltons
Estimated glomerular sieving coefficient	0.01–0.10	Approximately 1.00
Method of elimination	Glomerular filtration, 100% reabsorption and catabolism by proximal tubules	Glomerular filtration, no reabsorption, active secretion by proximal tubules
Reference values	Infants/children	Infants/children
	0–3 mo: 0.81–2.32 mg/L	<2 y: 0.4–0.5 mg/dL
	4–11 mo: 0.65–1.49 mg/L	2–8 y: 0.5–0.7 mg/dL
	1–17 y: 0.50–1.27 mg/L	9–18 y: 0.6–0.9 mg/dL
	Adults: 0.59–0.91 mg/L	Adults: 0.8–1.4 mg/dL
Confounding variables	Determinants of general cellular metabolism	Muscle mass (determined by age, gender, race, strength training)
	Increase levels	Increase levels
	Corticosteroids early after renal transplant	Ketotic states, hyperglycemia (Jaffé)
	Hyperthyroidism	Cephalosporins (Jaffé)
		Flucytosine (enzymatic method)
		Cimetidine, trimethoprim (block secretion)
		Vigorous exercise
		Ingesting cooked meats
		Decrease levels
		Dietary protein restriction
		Muscle wasting
		Malnutrition
		Bilirubin (Jaffé)
		Advanced age
		Female sex
		Advanced liver disease

system can operate properly. Alkali and alkaline earth metal hydroxides are typically used to maintain a suitably high pH in these reagent systems.

Cystatin C is a 122-amino acid, 13,250-Dalton protein that is a member of the family of cysteine proteinase inhibitors.[11] It is the product of a "housekeeping" gene expressed in all nucleated cells and is produced at a constant rate. Because of its small size, cystatin C is freely filtered by the glomerulus. It is not secreted but is reabsorbed by tubular epithelial cells and subsequently catabolized so that it does not return to the blood flow (Table II). This latter property negates calculation of a cystatin C clearance using urine concentrations. The use of serum cystatin C to approximate eGFR is based on the same logic as the use of blood urea nitrogen and creatinine, but because it does not return to the bloodstream and is not secreted the eGFR obtained is believed to be more reflective of actual renal filtration function.

Endothelial cells, podocytes, mesangial cells, and the glomerular basement membrane (GBM) are all components of the glomerular membrane and are important for its function. Properties of endothelial cells and their surface layer, the GBM, and podocytes account for variations glomerular permeability and analyze data concerning the restriction of solutes by size, charge, and shape. The glomerular barrier is highly size- and charge-selective, in qualitative agreement with the classical studies performed 30 years ago. The cellular components are the key players in restricting solute transport, while the GBM is responsible for most of the resistance to water flow across the glomerular barrier. The sieving coefficient of a solute is a measure of its transport rate in relation to that of water. Interactions between the particle and the wall are mediated by viscous stresses and pressure variations in the fluid. Such hydrodynamic interactions also cause the velocity of a freely suspended particle to

deviate from the mean fluid velocity. The finite size of the particle limits the positions that its center can occupy, bringing steric considerations into the calculation of clearance from the blood pool into the urinary space. Animal studies have shown that the reduction in angiotensin II caused by the initiation of an ACEI (and presumably angiotensin II receptor antagonists, direct renin inhibitors) reduces efferent arteriolar pressure and thus reduces intraglomerular pressure by reducing the transcapillary hydrostatic pressure along the glomerular capillary.[12] In addition, there is a small reduction in tubular secretion of creatinine into the urinary space and an enhanced proximal tubular reabsorption.[13] These mechanisms account for the predictable approximately 15% to 30% rise in serum creatinine in patients taking drugs that antagonize the RAS. It is conceivable that creatinine and cystatin C have differing permselectivity functions according to reduced intraglomerular pressure, which account for the wide discrepancy reported in the case. The relative size of creatinine is sufficiently small for it to have a high sieving coefficient; thus, it would have a greater reduction in filtration in response to a drop in intraglomerular pressure.[14] Cystatin C, a larger protein, is probably situated at a different position on the sieving function curve and is expected to be influenced less by a reduction in intraglomerular pressure with RAS-blocking drugs.[15] Other proteins with similar sieving coefficients to that estimated for cystatin C (0.01–0.10) and much lower that that of creatinine (approximately 1.0) include 1-acid glycoprotein and thyroid-stimulating hormone, both of which have never been described to change with a reduction in intraglomerular pressure with an RAS blocker.[16] In other words, creatinine, which is much smaller than cystatin C, is more easily pushed across the glomerular barrier into the urinary space, so a drop in glomerular pressure with an RAS blocker results in less filtration of creatinine. Conversely, serum cystatin C achieves a steady state in the blood, is less responsive to fluctuations of intraglomerular pressure, and may not change with the introduction of an RAS blocker. Thus, it is possible that the cystatin C level and eGFR estimates were entirely normal in this patient because true glomerular filtration function was preserved and remained stable for cystatin C. This is an important concept for individuals with lower eGFR ($<$60 mL/min/1.73 m^2) based on serum creatinine while taking RAS blockers who could be misclassified as having CKD.

Sebekova studied 12 individuals with newly diagnosed nondiabetic kidney disease (tubulointerstitial nephritis, n=8; glomerulonephritis, n=2; polycystic kidney disease, n=2) and impaired renal function, who were subsequently treated with the ACEI ramipril 5 mg po qd for 2 months and found changes from baseline to follow-up of 1.93 to 1.91 mg/dL in serum creatinine and 1.68 to 1.65 mg/L in cystatin C, while measured creatinine clearance decreased slightly from 0.72 to 0.76 mL/s, suggesting no differential effect when creatinine clearance is unchanged.[17] In a study by Watanabe and associates,[18] 30 nondiabetic hypertensive patients treated with the angiotensin receptor blocker valsartan for 3 months had decreases in serum cystatin C (0.63 ± 0.31–0.52 ± 0.27 mg/L; P=.015), but not in serum creatinine (0.75 ± 0.15–0.74 ± 0.18 mg/dL; P=.712). The renal resistive index by Doppler reduced, suggesting differential changes in renal filtration of creatinine and cystatin C when treated with an angiotensin receptor blocker, which caused reduced intraglomerular pressure. Several studies have shown that the eGFR derived from cystatin C equations are more accurate than creatinine equations when a reference standard such as iohexol clearance is measured; however, none have specifically evaluated those treated with RAS blockers.[8,19,20] A recent report found that when compared to eGFR determined by urinary clearance of 125I-iothalamate and 51Cr-EDTA, cystatin C was 4.3% lower for every 20 years of age and 9.2% lower for female sex, but only 1.9% lower in African Americans.[21] Thus, these data suggest that cystatin C is slightly lower in those with reduced overall cellular mass and is influenced to a much lesser degree than serum creatinine. We recognize that cystatin C is not the only new biomarker giving inferences on acute kidney disease and CKD and that novel measures including the renal expression of neutrophil gelatinase lipocalin, kidney injury marker 1, soluble sodium-hydrogen exchanger 3, and interleukin 18 and sophisticated measurement of iohexol clearance may be future approaches, in addition to the current tools available, to understand whether true kidney disease is present in patients with an elevated serum creatinine level on an RAS blocker.[22]

This case report has all the limitations of single observations but is unlikely to be confounded by measurement issues for either serum creatinine or cystatin C. Of importance, we cannot make inferences about normal individuals not on RAS blocker therapy. A gold standard for assessing renal filtration function such as insulin clearance was not performed. However, the wide disparity between eGFRs calculated using serum creatinine and cystatin C suggest that RAS blocker therapy may

be a special circumstance in which cystatin C is a preferred test for assessment of renal function.

In conclusion, the determination of normal renal filtration function in a patient who is treated with drugs that block the RAS (ACEIs, angiotensin receptor blockers, direct renin inhibitors) can be difficult given the expected rise in serum creatinine. Cystatin C may prove to be a useful measure in patients taking these medications as a more reliable and accurate reflection of true glomerular filtration function.

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